Fertilization, Implantation, and Early Embryogenesis

Introduction

This lesson is about fertilization of the oocyte within the uterine tubes, implantation of the embryo into the uterine lining, and formation of the placenta. We are going to skip a BUNCH of stuff. The illustrations include a lot of this skipped material and can be used in conjunction with more specialized texts, or they can serve as points of departure for in-depth conversations with your professors if you are extra curious. But for now, we encourage you to learn what we teach you, which is enough to understand how the placenta works and how it relates to ovarian cancers. These may seem like completely disconnected concepts, but to talk about ovarian cancer, you must first understand implantation and the activity of the placenta.

We start with capacitation and fertilization, highlighting the changes that occur in the spermatozoa and changes in the oocyte after it has been fertilized by a spermatozoon. We then mention, but more or less gloss over, morulation and blastulation (do not spend time learning these). We end with a detailed discussion of the fetal-placental unit and how the placenta and maternal circulations do not mix but enable the developing embryo to grow into a fetus and, eventually, a neonate.

But between fertilization and the fetal-placental unit, we follow the development of the embryonic and extraembryonic structures—the amnion, chorion, umbilical cord, and decidua. Do not worry about seeing those words here in the introduction. We are going to guide you through this material in a manner that isn't just memorizing and regurgitating. The names of structures change and are often unnecessarily long, but these are the words that define these processes.

Medical science frustratingly uses the word embryo to mean both the fertilized zygote and the thing that becomes the baby. We want you thinking zygote, embryo, fetus, neonate—everything else is cut off and thrown away. Technically, everything extraembryonic (not the embryo/fetus/neonate) *is* part of the "the embryo," having all been derived from the one fertilized egg. **This is not an ethical discussion, only a means for clarity.** At OME, when we say "embryo," we mean everything other than the stuff that is clamped, cut, and removed from the healthy neonate. The embryo-derived neonate goes home with mom. Everything else—chorion, amnion, yolk sac—that is derived from the fertilized ovum but clamped off, cut, and sent to be disposed of (aka the placenta) is *not* the embryo. We use **embryo** to specifically refer to the thing that is not discarded after delivery, and we use **conceptus** to mean "all the things," including the embryo, placenta, and all extraembryonic membranes. Everything that is made from the fertilized egg (the zygote), whether it becomes placenta or embryo—the entire unit—we refer to as conceptus.

Capacitation and Fertilization

The spermatozoa undergo a process called **capacitation**, a physiological process that is necessary for fertilization, but the mechanisms of which are poorly understood. This process happens in the female reproductive tract. Researchers have extracted spermatozoa from the epididymis and implanted them in the uterine tube or near the ovary. Anywhere the spermatozoa are placed results in fertilization, meaning that there isn't something that happens in the male genital tract, recipient vagina, cervix, or uterus. How the spermatozoa know they are in the female reproductive tract and that they should undergo capacitation remains uncertain to medical science.

What is known is each of three specific events: hyperactivation via calcium channels, activation of the acrosomal cap, and ZP3.

Spermatozoa can be identified as **hyperactivated** when their flagella become erratic and more forceful. This is achieved with the insertion of **calcium channels** in the plasma membrane in the **tail region**.

More calcium, more forceful contraction, more movement—the spermatozoa move faster and have more penetrating force. It is thought that this is what enables the spermatozoa to penetrate the corona radiata, to get through the granulosa cell layer (the layer that protects the oocyte and prevents the second meiotic division, holding the oocyte in meiosis 2).

When the spermatozoa reach the zona pellucida, the glycoprotein **ZP3** (zona pellucida glycoprotein 3) acts as a receptor for some ligand on the heads of the spermatozoa. ZP3 enables spermatozoa to bind to and remain near the zona pellucida. After this attachment, **vesicles** of enzymes are released between the spermatozoa and the zona pellucida; these enzymes degrade the glycoproteins of the zona pellucida, eroding a tunnel for the spermatozoa.

With a forceful contraction of the tail, the plasma membrane of one spermatozoon fuses with the plasma membrane of the oocyte. This fusion results in the nucleus in the head of the spermatozoon being injected into the cytoplasm of the oocyte (strongly resembling a virus, the sperm acting as a virion).

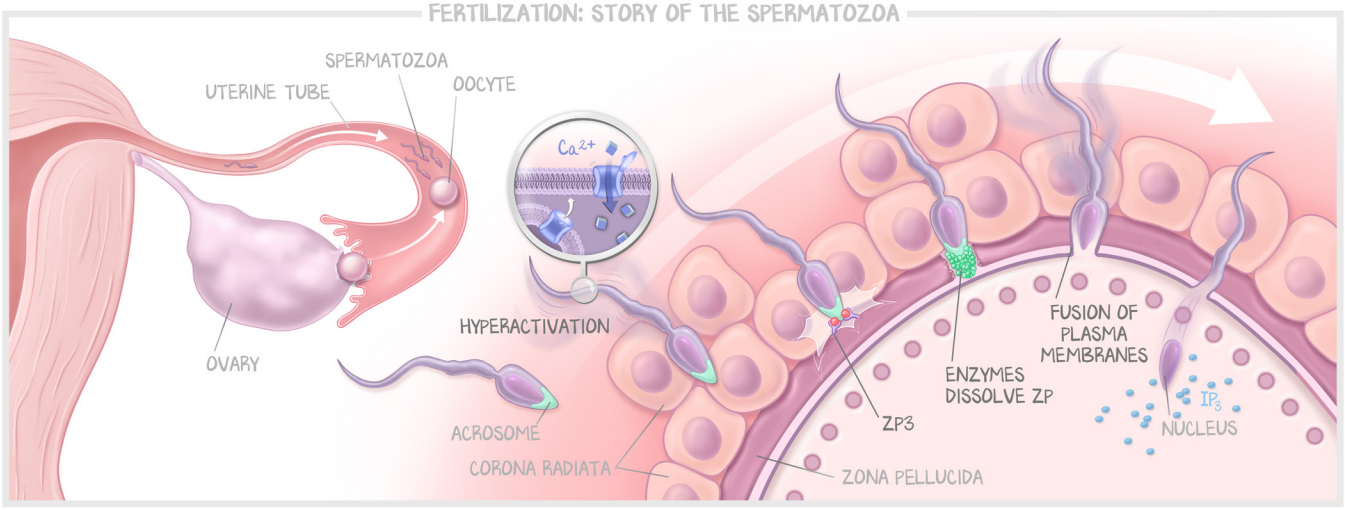

Figure 5.1: Fertilization: Story of the Spermatozoa

The purpose of the corona radiata and zona pellucida is not to ensure that only the strongest spermatozoon wins its prize. Instead, they are present to ensure that one and only **one spermatozoon's** DNA makes it into the oocyte cytoplasm. At the same time as the injection of the nucleus, the spermatozoon also infuses the oocyte cytoplasm with **IP_3**. IP_3 is the second messenger associated with G_q-IP_3-Ca^{2+}. Only instead of G_q making the IP_3, which then releases the calcium from the endoplasmic reticulum, the spermatozoa have a bunch of premade IP_3 ready to go. When a spermatozoon fuses, all of its premade IP_3 is dumped into the oocyte cytoplasm, and all of that IP_3 induces calcium to enter the cytoplasm from the endoplasmic reticulum. With a sudden rush of cytoplasmic calcium, the oocyte depolarizes in a process lasting up to one minute.

That depolarization is the signal that tells the granulosa cells of the corona radiata that their work is done. The granulosa cells of the **corona radiata release** their cytoplasmic connections, release their inhibitory control of meiosis, which permits the completion of meiosis 2. One oocyte undergoes two divisions, resulting in the combination of one haploid nucleus from the oocyte with the spermatozoon's haploid nucleus and the presence of three polar bodies—retired genomes that never become cells. The depolarization also causes a **cortical reaction**, in which granules, already placed by the oocyte at its cytoplasmic periphery (just barely within the plasma membrane, all around the entirety of the perimeter), fuse with the plasma membrane, instantly increasing the barrier thickness of the zona

pellucida. The remaining spermatozoa are not only stunned by the depolarization but also suddenly have double the distance to tunnel through. This provides the time necessary for the oocyte to complete meiosis 2 and become the mature ovum, as well as the marrying of the haploid nuclei to form one diploid nucleus. With degeneration of the nuclear membrane (not fusion), the chromosomes pair for the first mitotic division. When the two sets of haploid DNA combine to become diploid, fertilization is finished, and embryogenesis has begun.

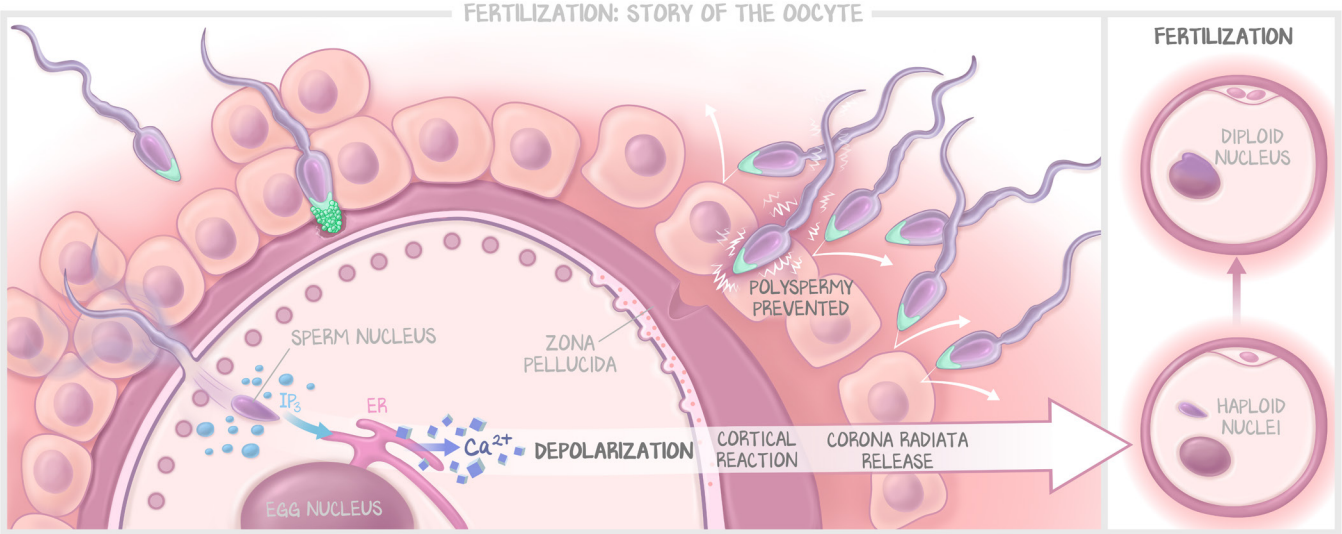

Figure 5.2: Fertilization, Story of the Oocyte

Fertilization to Implantation—Early Blastocyst Formation

This section uses words like "morula" and "blastocyst." You need the information in this section to follow through the rest of early embryogenesis, but you don't have to get caught up on the details. What you need to get is *"trophoblast = outer cell layer; embryoblast = inner cell layer; embryoblast is a hypoblast and epiblast."*

The fertilized egg, the zygote, undergoes a mitotic division into two cells. Those two cells divide, so now we have four cells. Those four cells divide to become eight cells, and the eight cells divide to become a sixteen-celled morula ("morula" is just the name of the sixteen-celled thing). The morula is important because, until this point, the zona pellucida persisted; therefore, although the embryo was increasing in cell count, the overall structure didn't change. As the 16 cells become 58 (the numbers are no longer important, this is just in keeping with our reference embryology book), the zona pellucida degrades, and the pre-implantation conceptus becomes a 58-celled blastocyst ("blastocyst" is what the 58-celled thing without a zona pellucida is called). Fluid enters the blastocyst through the degraded zona pellucida, forming the blastocyst cavity. Fluid enters the blastocyst through the degraded zona pellucida, forming the **blastocyst cavity**. There are two types of cells in the blastocyst: those that form a ring around the outside, called the outer cell layer, and those that form a cluster contained by that outer cell layer, called the inner cell layer. The outer cell layer surrounds the blastocyst cavity and the inner cell layer. The cells of the inner cell layer stay clustered together, polarizing the blastocyst. Where the inner cell layer is located relative to the blastocyst cavity is the embryonic pole. We present the inner and outer cell layers to set you up for success in understanding what comes next. Notice how little was bolded. The purpose of this section was to allow your brain to accept: "fertilization is complete, now we have a ring of cells around a fluid-filled cavity and another cluster of cells" (see Figure 5.3).

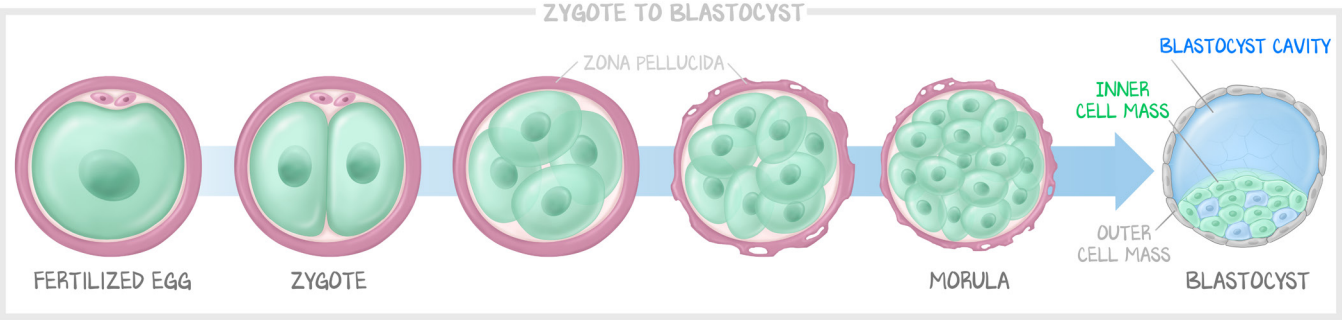

Figure 5.3: Zygote to Blastocyst

If fertilization occurs at the right time (in the ampulla of the ovarian tube), then as the conceptus arrives in the uterine cavity, it will have reached the blastocyst stage, and it is ready to implant. It is also capable of secreting **human chorionic gonadotropin (hCG)**. hCG stimulation is required for the **persistence of the corpus luteum**, which in turn continues to produce progesterone, silencing any FSH signal from the anterior pituitary and enabling the uterus to maintain its secretory phase. The absence of FSH means new follicles won't enter the growth phase. A prolonged secretory phase gives the uterus time to develop a rich endometrium, into which the placenta can burrow.

The **outer cell layer** becomes the **trophoblast** (the cytotrophoblast and the syncytiotrophoblast), which develops into the **chorion** and **blood villi** of the placenta. But before the trophoblast does this, it interacts with the uterine endometrium. Where the trophoblast touches the endometrium, the trophoblast cells divide and differentiate a daughter cell to become a **syncytiotrophoblast** cell. The trophoblast continues this process, adding more and more syncytiotrophoblasts to the syncytium. These syncytium-forming cells dissolve their own plasma membranes, coalescing into a multinucleated glob that eats away the endometrium (i.e., making a syncytium). This amalgam of nuclei burrows into the endometrium, making room for the conceptus and searching for blood and nutrients. Where there is no direct overlap between the outer cell layer and the endometrium that it bores through, the trophoblast cells divides and differentiates into two **cytotrophoblast** cells. The outer cell layer forms the **chorion**.

The **inner cell layer** becomes the **amnion** (amniotic cavity, which contains amniotic fluid), **yolk sac**, and **embryo**. We can't say the outer cell layer is the placenta, and the inner cell layer is the baby. Instead, we have to say everything that is of the embryo—embryonic, intraembryonic, the thing that becomes the living organism outside the womb—is the embryo, and everything else is not. The yolk sac, amnion, chorion, and umbilical cord are all **extraembryonic**. But only the chorion comes from the outer cell layer, whereas everything else—amnion, yolk sac, umbilical cord, and embryo—is derived from the inner cell layer. To generate the yolk sac, amnion, and embryo, the inner cell layer organizes itself into two layers, the hypoblast and epiblast. The **hypoblast**, which is in contact with the blastocyst cavity, proliferates to envelop the blastocyst cavity and become the yolk sac (also known as extraembryonic endoderm). The **epiblast layer** is oriented closest to the embryonic pole; it proliferates to form the amniotic cavity and also becomes the trilaminar disc—the source of embryonic ectoderm, endoderm, and mesoderm—which becomes the embryo.

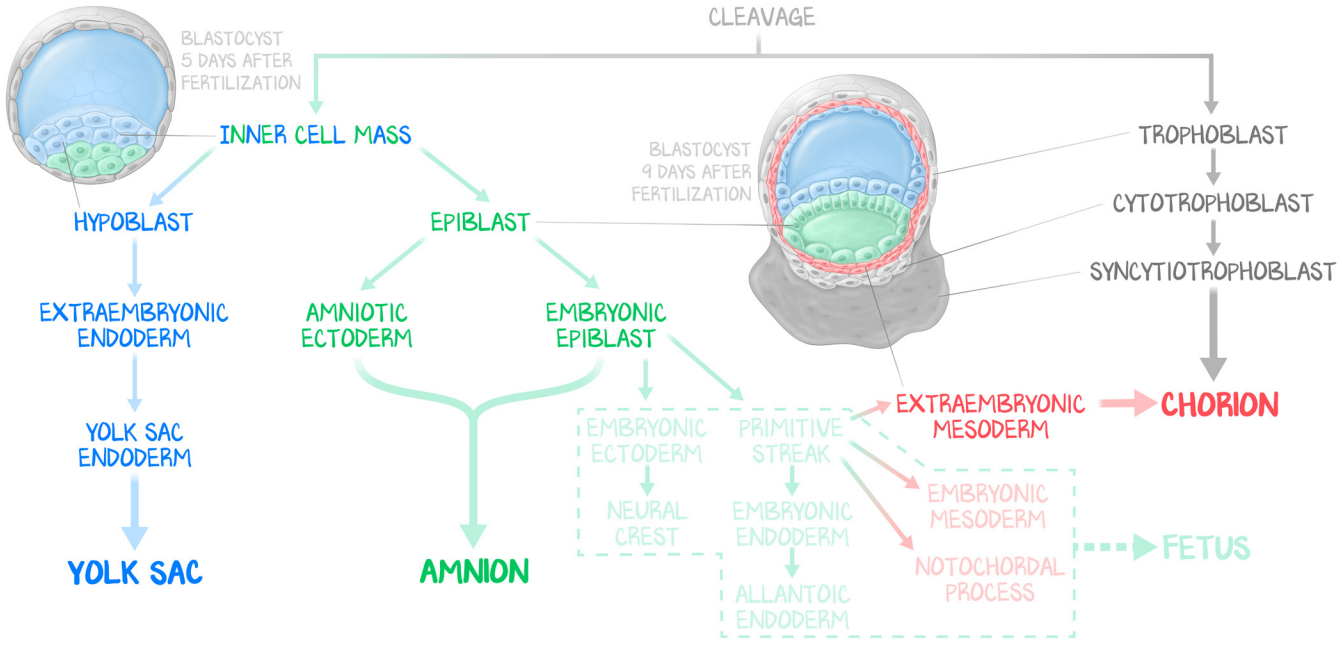

Figure 5.4: Destiny of Blastomeres

We're going to take a nomenclature shortcut. In this lesson, we do not care about the embryo (every other module did that already). That means there are only three main structures to identify and learn, which neatly fit with three origins. The **epiblast** becomes the **amniotic cavity**, the **hypoblast** becomes the **yolk sac**, and the **trophoblast** becomes the **chorion** and **placental blood vessels**. We can also use the parent-daughter relationship to easily communicate the level of detail we mean. For example, when we mean the entire outer cell layer and what it becomes, we use "trophoblasts," which is inclusive of the cytotrophoblasts and syncytiotrophoblasts. When we want to be more specific, as when we want to talk about the syncytiotrophoblasts eroding the endometrium, we use the more specific term (syncytiotrophoblasts). If you ever feel lost in nomenclature, come back to this figure.

As with much of the confusing language in medical sciecnce we want to take a moment to define what we're doing and also what you might see elsewhere. Some texts refer to the syncytiotrophoblast as a tissue, the entire amalgam of syncyctiotrophoblasts. We want you thinking in terms of cells and cell divisions. The syncytium of syncytiotrophoblasts increases in size when cytotrophoblasts divide and differentiate a daughter to become a syncytiotrophoblast, a cell. Just like we did with the word 'embryo,' we are going to keep this cell focused. The trophoblast will become one of two cell types, the cyto-trophoblasts and the syncytio-trophoblasts. The cells that make up the syncytium are the syncytiotrophoblasts. We'll use syncytiotrophoblast to mean an individual nucleus and its cytoplasm as it joins the syncytium, and syncytiotrophoblasts to mean all the nuclei and all the cytoplasm of the syncytium, acting in concert.

Trophoblast to Chorion, Hypoblast to Yolk Sac, Epiblast to Amniotic Cavity and Embryo

The trophoblast is going to be doing something at the same time that the epiblast and hypoblast are doing something. The illustrations in this section are arranged in chronological order. The paragraph before the illustration will be the text for the trophoblast. The paragraph after the illustration will be the text for the epiblast and hypoblast. Read the trophoblast paragraph, then map it on the figure. Read the epiblast/hypoblast paragraph and map it on the figure. Then review the figure a third time, tracking them all together. Only then move on to the next illustration's trophoblast paragraph, repeating the process for each illustration.

The uterine epithelium is simple columnar epithelium that invaginates into glands that, now in the secretory phase of the uterus, are engorged by their own secretions. The stroma is likewise rich in vasculature. The cells of the trophoblast that touch the endometrium divide and differentiate their daughter cells into **syncytiotrophoblast** cells. The syncytiotrophoblasts erode the uterine epithelium and underlying stroma in search of nutrients and blood vessels. Simultaneously, the syncytium enlarges as the trophoblast continues to divide and differentiate daughter cells, and the entire conceptus begins to sink into that eroded endometrium, following the path the syncytiotrophoblasts have dug. As the syncytiotrophoblasts melt away the underlying tissue and the embryo follows, the cytotrophoblasts proliferate to accommodate the growing inner cell mass. Eventually, the entire conceptus, including the cytotrophoblast layer, sinks so deeply into the endometrium that it is below the surface epithelium of the endometrium. The stromal cells, the glycogen-rich **decidual cells,** then close in the conceptus beneath the epithelium.

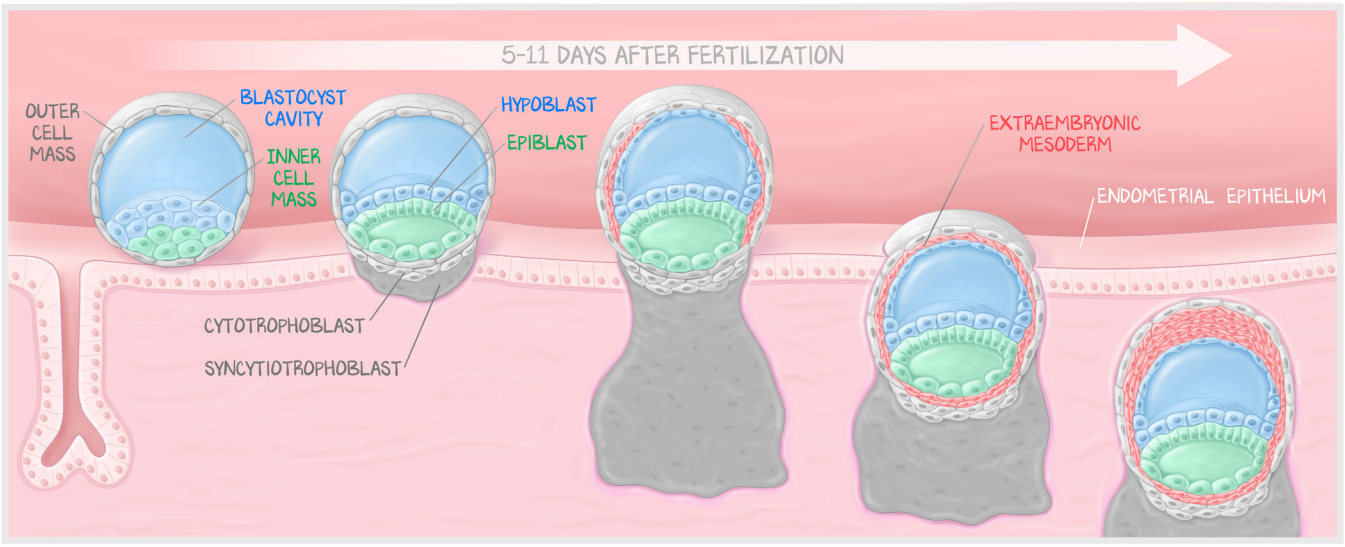

Figure 5.5: Implantation

The **hypoblast** proliferates and takes control of the **blastocyst cavity**. The **epiblast** divides and differentiates a daughter cell to become the **amnion**, the epithelium of the **amniotic cavity.** The epiblast also becomes the trilaminar disc of the embryo (though we are not following the embryo). The epiblast also divides and differentiates daughter cells to become **mesoderm**. The conceptus enlarges during this time, mainly because of the mesodermal proliferation. All around in every direction, **extraembryonic mesoderm** proliferates between the trophoblast and the inner cell mass, driving them apart (this is also when the intraembryonic mesoderm forms the trilaminar disc, but embryonic development is not the focus of this discussion). So, as the conceptus bores its way into the endometrium, the conceptus enlarges and fills with mesoderm.

As the mesoderm proliferates, the syncytiotrophoblasts continue to drill into the endometrium, finding blood vessels and endometrial glands rich in nutrients, and the cytotrophoblasts proliferate to accommodate the expanding mesoderm. Extraembryonic mesoderm proliferation continues, but **gaps in the mesoderm** begin to form. These gaps are mesothelial-lined, fluid-filled cavities. As more and more form, they soon coalesce to from the **extraembryonic coelom**—the Body Cavity outside of the embryo. There is also an intraembryonic coelom that is the First Body Cavity that becomes all Body Cavities. This extraembryonic coelom is not that. At this stage in development, there is hardly an embryo to speak of. The extraembryonic coelom is the cavity between the inner cell mass and the trophoblast. Thus there is a polarity to the extraembryonic coelom's mesothelium—a trophoblast layer and an inner cell layer. Of

course, these layers are continuous with one another, just as we debunked parietal and visceral layers of Body Cavities. But this visualization will make your life much easier. A small layer of mesoderm remains between the mesothelium of the extraembryonic coelom and the trophoblast, as well as between the mesothelium of the extraembryonic coelom and the inner cell mass. The mesoderm in contact with the hypoblast and epiblast is called extraembryonic **splanchnic** mesoderm, and it becomes the amnion and yolk sac. The mesoderm in contact with the trophoblast is extraembryonic **somatic** mesoderm, and it becomes the chorion.

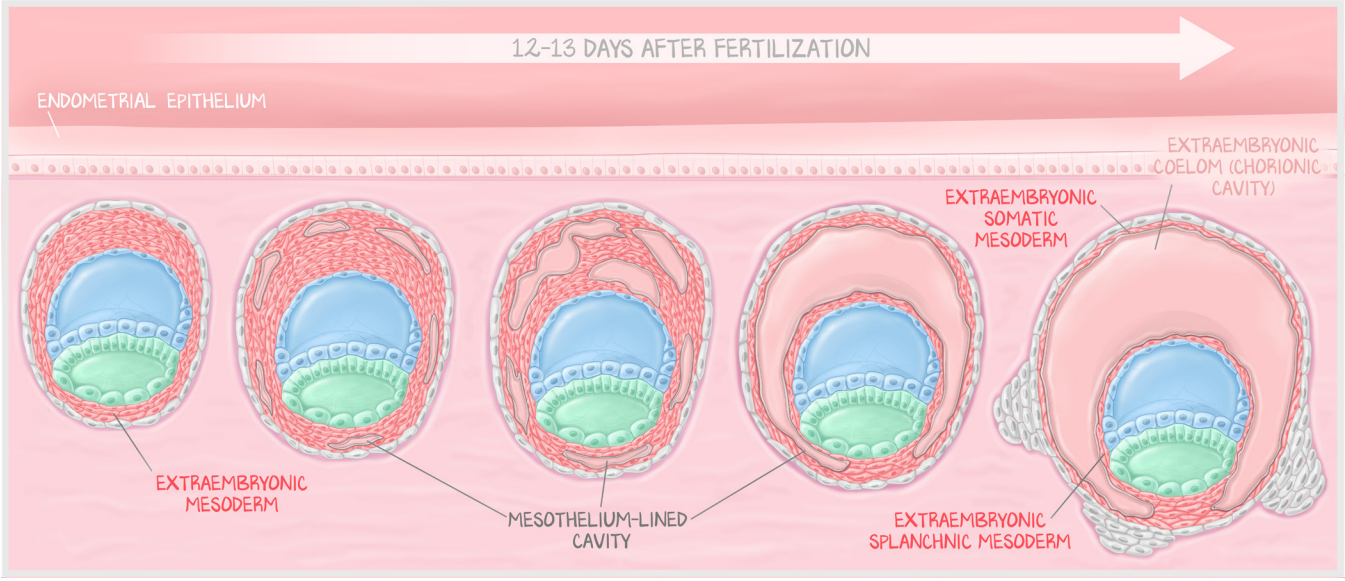

Figure 5.6: Formation of the Extraembryonic Coelom

While the extraembryonic coelom is being formed, the hypoblast forms the **yolk sac,** and the epiblast proliferates and generates the **amniotic cavity** and **embryo**. The only connection between the extraembryonic splanchnic mesoderm and the extraembryonic somatic mesoderm is the mesoderm of the **connecting stalk**. This mesoderm bridges all of the structures of the developing conceptus—where the yolk sac, amniotic cavity, embryo, and chorion come together. The connecting stalk is the mesoderm that becomes the **umbilical cord**.

The cytotrophoblasts, their somatic mesoderm, and the mesothelium that lines the extraembryonic coelom are together called the **chorion**, and the extraembryonic coelom is synonymous with the **chorionic cavity**. The chorion excludes the syncytiotrophoblast. How the trophoblast establishes the maternal-placental exchange is covered in the final section of this lesson. It requires more than just a single paragraph. This part is intended to set you up for the next section, which explores the destiny of the chorion as the pregnancy progresses. Because no blood vessels have been established, there is no heart, and we are still before the sixth week in development. The chorionic cavity is the space for the yolk sac, amniotic cavity, and embryo to grow into.

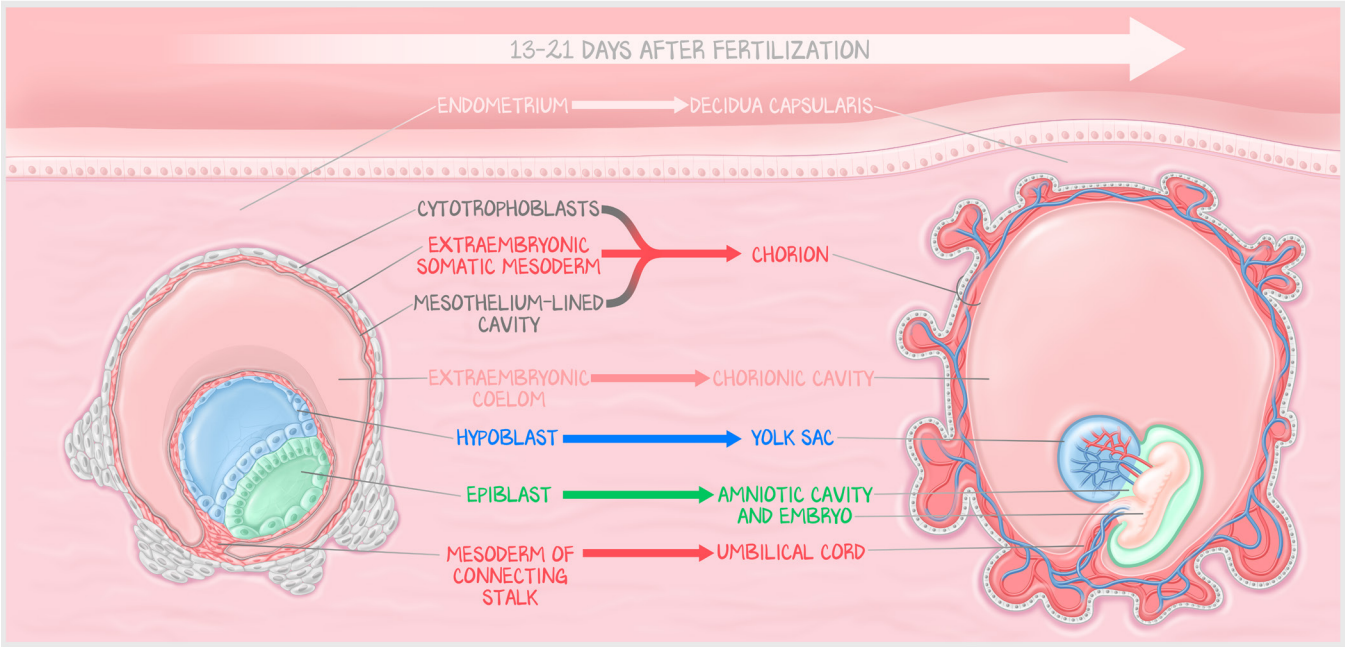

Figure 5.7: Primitive Streak Forward

The **amnion** is the epithelium of the **amniotic cavity**. The amnion epithelium is derived from and continuous with the epiblast. The epiblast that is not the amnion becomes the embryo; the epiblast that is the amnion surrounds the embryo and cushions it throughout the pregnancy. The amniotic cavity is filled with fetal urine, which provides a fluid cushion for the fetus. The **yolk sac** is initially crucial to the development of the embryo, providing extraembryonic endoderm. As the embryo grows, the yolk sac does not.

Placental Membranes

The chorion initially forms as a spherical structure surrounding the embryo within the uterine wall, with blood villi in all directions, the chorionic cavity surrounding the inner cell mass. As the embryo and amnion grow, they fill the chorionic cavity and eventually push out from beneath the surface epithelium of the uterus into the uterine lumen. There will always be a layer of uterine endometrium (the decidua), a layer of chorion, a layer of amnion, and then the embryo. But as the embryo becomes a fetus, it continues to enlarge. The once fist-sized uterus swells during the duration of pregnancy.

Where the chorion maintains its villous connections with the uterus, it remains thickened, has many villi, and is not smooth. Where the chorion is stretched by the embryo and pushed into the uterine lumen, it becomes stretched out, thinned, and smooth from loss of its villi.

As the embryo grows, so too does its production of amniotic fluid, which fills the amniotic cavity. Together, the amniotic cavity and the embryo-turned-fetus grow to occupy more and more of the chorionic cavity, likewise displacing the yolk sac. The fetus floats in and is cushioned by the amniotic fluid, which protects the fetus while allowing the mother to remain active.

The endometrium being pushed into the uterine lumen and in contact with the smooth chorion is called the **decidua capsularis**. The endometrium that is not in contact with the chorion is called the decidua parietalis. The decidua is the endometrial reaction to implantation, where stromal cells differentiate into decidual cells. That's literally all we need you to know about them. The transition from decidua capsularis to decidua parietalis is termed decidua basalis.

As the fetus gets even larger, the uterine lumen is likewise filled. The decidua-lined chorion gets compressed against the posterior uterine wall. In a normal pregnancy, the chorion is smoothest and smallest at the top of the fetus's head, aimed down, pointed at the cervix. Above the chorion—between the internal os and the chorion—is whatever small remnant of decidua capsularis is left. Below the chorion is the amnion and the amniotic fluid it contains.

The event colloquially referred to as having a woman's "water break" is called the "**rupture of membranes**" in medicine. The **membranes** are the **decidua capsularis**, the **chorion**, and the **amnion**. All three are extremely thin by this time, so a rupture of one membrane generally means a rupture of all three. The rush of fluid is the amniotic cavity emptying its contents, losing the buoyant cushion baby developed in. Now, the way out is clear, and mom's uterine contractions can begin to expel baby. All the while, baby is still getting nutrients from mom via the placental blood villi. More on delivery in the Pregnancy and Delivery island.

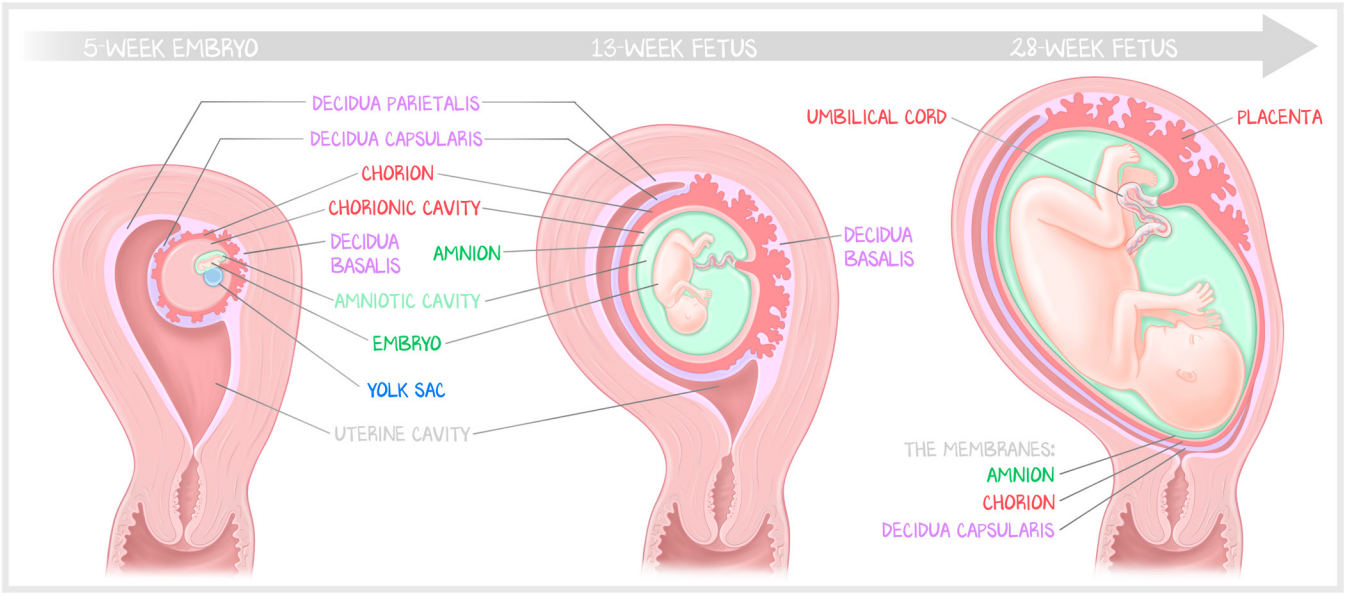

Figure 5.8: Final Placental Membranes

Formation of the Chorionic Villi

Because the chorionic villi formations are so complex, we saved it for the very end. We wanted to show you that the earliest structures were worth learning because they become the final placental membranes and that they aren't just the subject of an esoteric embryologic discussion, something that involutes by week 6. But now we have to go back to early embryogenesis, weeks 3–5, where the chorion forms blood villi and establishes the placental-maternal blood exchange.

The **chorion** is the cytotrophoblast and the underlying mesoderm outside the chorionic cavity. The chorionic cavity is the space that the embryo, yolk sac, and amniotic cavity grow into. The **chorionic plate** is a mass of mesoderm that encircles the chorionic cavity and is synonymous with extraembryonic somatic mesoderm. The mesoderm of the chorionic plate is contiguous with the mesoderm of the embryo only at the **body stalk**. When that mesoderm becomes blood vessels, the blood vessels of the chorion become contiguous with the blood vessels of the embryo, through the **umbilical cord**.

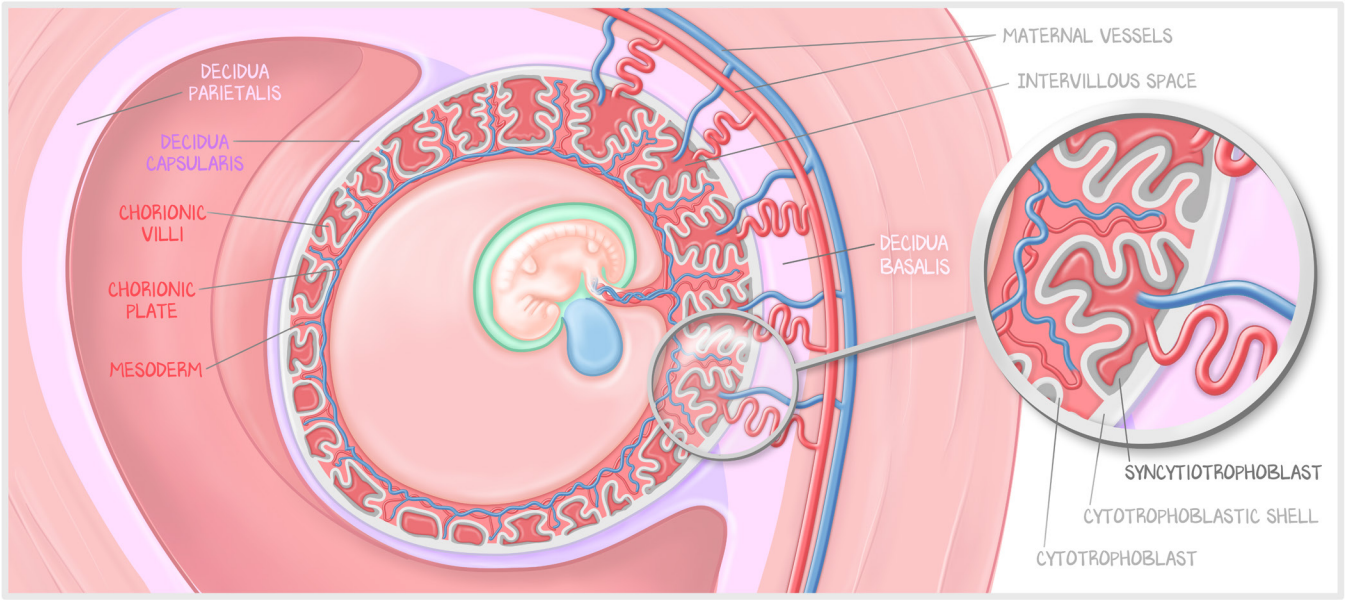

Figure 5.9: Membranes of the Placenta

The cytotrophoblasts are the external lining of the chorion and line the chorionic villi as well. The cytotrophoblasts had been dividing and differentiating daughter cells into the syncytium that was busy invading the endometrium. The syncytiotrophoblasts erode the endometrium until they find maternal blood vessels. They then form hollowed structures lined with syncytiotrophoblasts that are continuous with the maternal blood flow. From uterine arteries into these cavernous spaces then back out through uterine veins.

The chorion sends out **primary villi** from the chorionic plate. These primary villi are made up of cytotrophoblasts. These cytotrophoblasts use the syncytiotrophoblast as a scaffolding, growing out towards the periphery of the syncytiotrophoblasts. The cytotrophoblasts climb out over the syncytiotrophoblasts to form an **outer cytotrophoblastic shell** with a cytotrophoblast epithelium several cytotrophoblasts thick. As the syncytiotrophoblasts are replaced by cytotrophoblasts, the cytotrophoblasts form an epithelium just behind the syncytiotrophoblasts. Thus, every blood-filled space is lined with a layer of syncytiotrophoblasts and a simple cytotrophoblast epithelium. The primary villi are termed **secondary villi** as the villi fill with **mesoderm** from the chorionic plate. This is, of course, continuous—as the cytotrophoblast epithelium advances over the syncytiotrophoblasts' syncytium, the mesoderm fills in the space left behind by the cytotrophoblast epithelium. **Tertiary villi** are the mature, terminal form of the chorionic villi. The event that changes the secondary villi to tertiary villi happens across the embryo and placenta all at once—the **mesoderm becomes blood vessels**. The extraembryonic mesoderm of the chorionic villi, the chorionic plate, the connecting stalk (which, once it has blood vessels, is renamed the umbilical cord), and the intraembryonic mesoderm of the embryo all at once become one enormous and continuous vascular network. Two umbilical arteries bring blood away from baby towards the chorionic villi, and one umbilical vein arises to bring blood back from the chorionic villi.

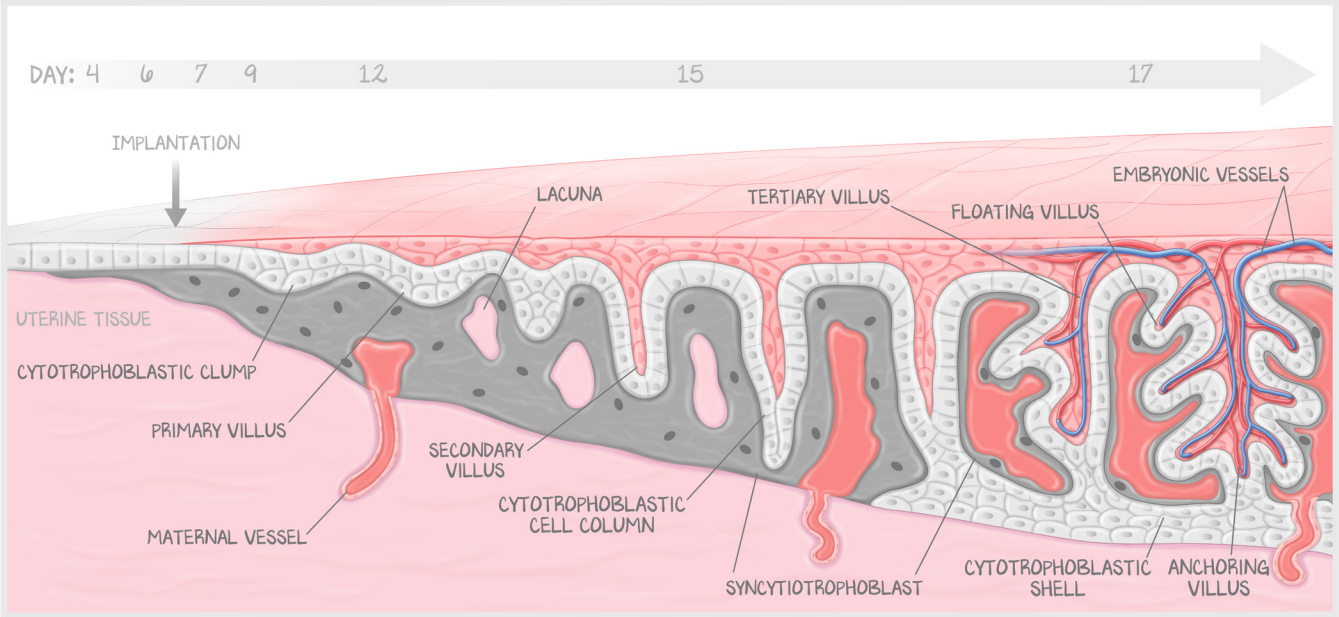

Figure 5.10: Chorion

The chambers left behind by the syncytiotrophoblasts are termed **intervillous spaces**. The only interruptions in the outer cytotrophoblastic shell are where **spiral arteries** or **uterine veins** penetrate into the intervillous spaces. By the time the mesoderm becomes blood vessels, the intervillous spaces are already continuous with maternal blood vessels. **The intervillous space becomes part of the maternal circulation.** When the mesoderm is triggered to form blood vessels (which happens across the embryo all at the same time), there has already been an established maternal flow into the intervillous spaces.

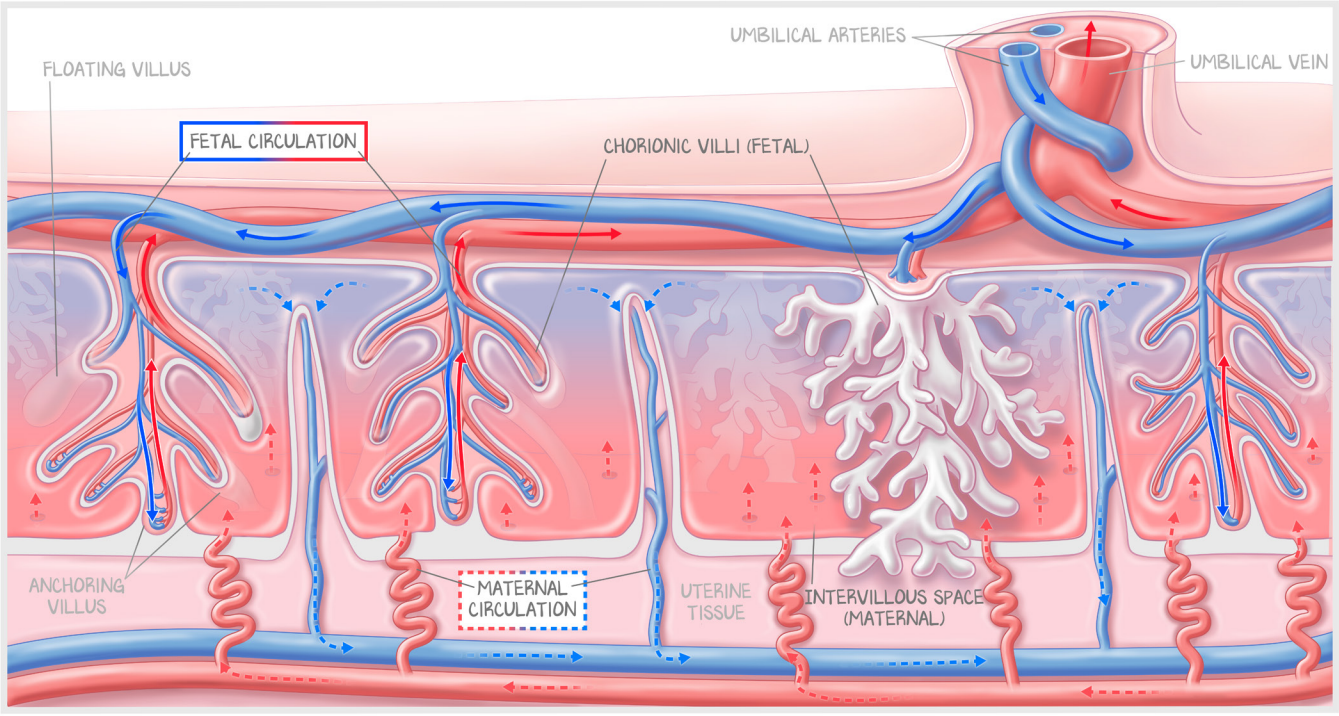

Figure 5.11: Chorion 2

Fetal and maternal blood never mix. The fetal blood stays within the villi. The maternal blood stays in the intervillous spaces. Both the syncytiotrophoblast layer and the cytotrophoblast epithelium prevent the mixing of blood but enable the exchange of gases (**oxygen into baby**, **carbon dioxide out**) and other nutrients. The placental-maternal exchange is not just a set of lungs; it is how mom is able to provide nutrients for the fetus. Proteins, lipids, ions, glucose, etc. must be able to get from mom's blood to baby's blood. In effect, the chorion is just another organ, connected to maternal circulation, harvesting maternal resources. The chorion then passes those maternal resources to the developing embryo. Mom's immune system is unaware of the fetus, which is kept far from maternal blood, encased in the chorion then amnion.

Conclusion

This lesson was necessary for us to discuss ovarian cancers. It is also a sneak peek at the Pregnancy and Delivery island. If you are left wanting on this subject, know that more information is on the way.